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Insidious weight gain in prepubertal seized rats treated with an atypical neuroleptic: The role of food consumption, fluid consumption, and spontaneous ambulatory activity

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ABSTRACT

Extreme obesity slowly develops in female rats over the months following seizures induced by a single systemic injection of lithium and pilocarpine if the resulting limbic seizures are treated with the atypical neuroleptic acepromazine (but not with ketamine). To discern the contributions from food consumption, water consumption, and (daytime and nighttime) activity to this weight gain, these behaviors were monitored for 4 months, about 2 months after seizure induction. The results indicated that the rats that underwent the obesity procedure exhibited 50% heavier body weights and consumed 42% more food than the reference group, which included rats that had been induced to seize but treated with ketamine. There were no statistically significant differences between groups with respect to either water consumption or (daytime or nighttime) activity. Factor analyses of data for individual rats verified the dissociation between activity and weight gain for the obese rats. The results suggest that the progressive weight gains are centrally mediated and are not secondary to diminished activity or altered fluid consumption.
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1. Introduction

Obesity has been considered an emerging global epidemic [1]. The reasons have been attributed to diet, diminished physical activity, and even the amount of food consumed in a nonsubsistence culture. Psychogenic nonepileptic seizures are also associated with an increased risk of obesity [2]. Nonepileptic seizures that exhibit the overt behavioral manifestations of epileptic seizures but are not associated with abnormal cortical discharges are more common than once assumed [3]. These observations suggest that neuroelectrical lability may be involved with the factors contributing to weight gain.

We [4] have been studying one experimental model of progressive obesity induced by administering acepromazine after the induction of limbic seizures in female rats with a single systemic injection of lithium followed 4 hours later by pilocarpine [5]. The obesity emerges in female rats that have undergone this obesity procedure before puberty [4]. Like the elicitation of weight gain by extended kindling [6] within the basolateral amygdala, the obesity subsequent to lithium/pilocarpine induction of seizures and treatment with acepromazine is strongly associated with the emergence of spontaneous seizures [4]. During the subsequent year, the body weight of a substantial number of these rats exceeds 1000 g.

The metabolic factors that contribute to the gradual weight gain are not clear. The most obvious candidates are (1) food consumption, (2) water consumption, and (3) activity, or various combinations. For example, chronic low-level diminished activity could contribute to weight gain, or minuscule but persistent increases in food consumption with normal activity could contribute to weight gain. This relationship may be relevant in light of the persistent elevation of nocturnal activity in male rats after they appeared to have recovered from lithium/pilocarpine-induced limbic seizures [7]. To discern empirically what variables might account for the heavier weights of the rats that underwent the obesity procedure, we designed the following experiment to monitor these variables.

Both this model of obesity and the goal of the present experiment are important for several theoretical and practical reasons. If neuroleptic drugs administered during specific developmental periods are contributing to obesity, then early identification may allow strategic intervention. Previous research has shown that the transient extreme hypothermia occurring in male rats induced to seize by this method, restrained, and given acepromazine was mediated by α_1-adrenergic receptors [8]. These receptors are

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located on adipocytes [9]. Acepromazine has also been shown to affect several receptors for different neurotransmitters [5,8] similar to some drugs employed as antipsychotic and antidepressant medications. Many of the subtypes of these receptors are involved with metabolism and eating.

2. Method

2.1. Subjects

In two separate blocks of experiments, a total of eight female Wistar albino (Charles River, Quebec) rats from four different litters were employed as subjects. Between 45 and 50 days of age, six of the rats were injected subcutaneously with 3 mEq/kg lithium chloride (3 mEq/cc) and then, 4 hours later, with 30 mg/kg pilocarpine (30 mg/cc). Within 30 minutes, the rats displayed rearing and forelimb clonus typical of Racine V convulsions [10]. Four of the rats received 25 mg/kg acepromazine. The combination of prepuberty, lithium/pilocarpine seizure induction, and postseizure treatment with acepromazine results in insidious weight gain and has been called, for convenience of expression, the obesity procedure. The range in body weights of the rats was 168 to 173 g at the time of seizure induction.

The two other seized rats, which were littermates of two of the four that received the acepromazine, received 100 mg/kg ketamine instead [11]. This procedure results in no weight gain and no emergence of overt spontaneous motor seizures. The other two rats, littermates of the remaining two seized rats given acepromazine, served as nonseized controls. Given the robust nature of the obesity effect [4], from this procedure and our data from two decades of measuring a large sample of normal rats over their life spans, we concluded that the anticipated very large effect size and the protracted periods of observation justified the small sample. After this procedure, the rats were maintained in standard plastic cages until the monitoring experiment began.

2.2. Procedure

At approximately 120 days of age, rats were housed singly in one of four custom-constructed plastic $32 \times 36 \times 45$-cm cages. The rats that had undergone the obesity procedure manifested gradual but increased body weight within 2 weeks of seizure induction and continued gaining weight faster than reference rats until they achieved the weights shown in Block 1 of Fig. 1. Food in the home cages was not measured before the experiments began.

In each cage a photocell placed midway along the width focused a light beam along the longitudinal axis. Interruption of the beam resulted in a recording. Numbers of interruptions were recorded in 5-minute blocks, 24 hours per day, for 126 days for each cage, for the duration of the experiments. Day- and nighttime activity measures were partitioned in the records. The light:dark cycle was 12:12, with local nocturnal conditions beginning at 20 hours. The baseline counts for the sensors were different within a factor of about 10.

Experiment 1 involved two rats that underwent the obesity procedure and two rats that were littermate controls. In Experiment 2, there were two rats that underwent the obesity procedure and two (littermates) that received the lithium/pilocarpine (seizures verified) but were then given 100 mg/kg ketamine. The duration of each experiment was approximately 4 months (126 days) over a 1-year period. During the period of continuous monitoring of activity, total amounts of food and water consumed were measured once every 2 to 3 days when the quarter-inch corncob bedding of the cages was changed. At that time, body weight was measured to the nearest gram.

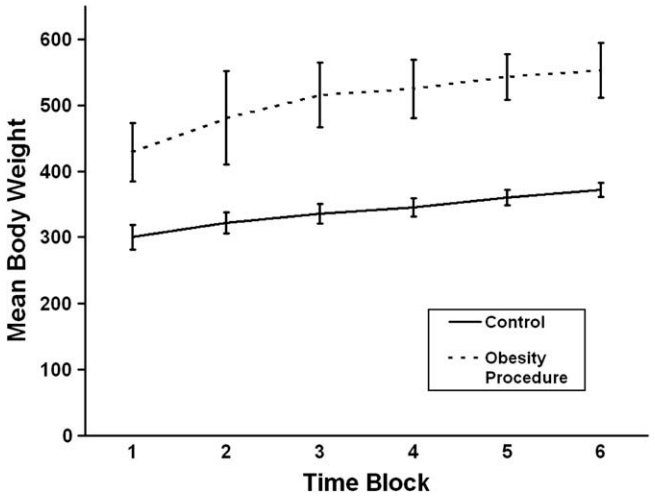

Fig. 1. Mean body weight (in g) of groups previously subjected to the obesity procedure (lithium/pilocarpine/acepromazine-treated rats) and controls (either lithium/pilocarpine/ketamine-treated or nontreated rats) over the six blocks (18 weeks). Vertical bars are SEM. Block 1 refers to 120 through 141 days of age, and Block 6, to 226 through 246 days of age.

Food (Purina Rat Chow cubes) consumption was calculated by subtracting the weight of food remaining from the weighed amount that had been placed in the cage previously. Another aggregate of carefully weighed food was placed on the fresh bedding. Care was taken to sort through corncob bedding to extract all discernable food residuals. Water was measured to the nearest cubic centimeter and discarded; water bottles were filled with fresh water. To standardize the values, the amounts of food and water consumed per day were calculated.

Two classes of analyses were completed. The first involved group analyses. To complete this procedure, mean daily body weight, daily food and water consumption, and daytime/nighttime activity for each rat were completed for successive intervals of 21 days so there were six blocks of measures. This block interval was selected so that the primary features of growth, rather than day-to-day variations, could be easily examined. Two-way analyses of variance with one within (repeated) and one between (group) level were completed for body weight, food consumption, water consumption, and activity (daytime and nighttime separately as well as nighttime/daytime activity ratios). To accommodate potential differences in the sensitivity (threshold) of the photocells for the different cages, within-subject z scores were calculated to detect any interactions between group and time.

To discern the presence of confounding variables, analyses of covariance were completed for each repeated measure over the six blocks by extracting residuals in symmetrical directions for successive pairs of variables. For example, residuals of body weight regressed with food consumption were completed for each block and then employed for the two-way analyses of variance. Then the reverse data, residuals of food consumption regressed with body weight, were employed for the analyses. The purpose was to find the causal source of any shared variance.

The second class of analyses involved daily body weights, food consumption, water consumption and daytime/nighttime activity for each rat singly. Partial correlations removing the contribution from activity were employed to ensure the relationship between successive weight gains (body weight – previous body weight, divided by the latter) and food consumption was evident on a "day-to-day" level. Factor analyses for these variables were completed (principal component, varimax rotation) for each rat separately to discern shared sources of variance for any intercorrelations.

All computations and analyses involved SPSS software on a Vax 4000 computer. Because of the robust nature of the effects, only a small sample size was required. Effect sizes (η^2), or how much variance in the dependent variable was accommodated by the treatment (group differences), were calculated to show the robust nature of the effects.

3. Results

3.1. Group analyses

Two-way analyses of variance for measures of body weight, food consumption, water consumption, nocturnal activity, daytime activity, and nighttime/daytime ratios over the six blocks as a function of the three groups (seized + acepromazine, i.e., the obesity procedure, seized + ketamine, nonseized controls) indicated no significant differences between the latter two groups. Consequently they were combined so there were only two groups: obesity procedure and controls. The six blocks referred to ages 120–141, 142–162, 163–183, 184–204, 205–225, and 226–246 days, respectively.

The mean (range) body weights for the entire duration were 339 (301–370) g for the reference group and 508 (421–587) g for the obesity procedure group. For food consumption per day, these values for the two groups were 24 (22–26) g and 34 (32–38 g), respectively. Mean water consumption per day was 42 (32–50) and 42 (36–46) cc, respectively.

The analyses (all $df = 1,6$) demonstrated that the obesity procedure group had significantly heavier body weights ($F = 16.16$). Group differences explained 72% of the variance in body weight. As shown in Fig. 1, there was a significant interaction between block (time) and group [$F(5,30) = 2.35$, partial $\eta^2 = 28\%$] as well as the main repeated measure effect ($F = 27.35$) for body weight over time.

Fig. 2 shows the significantly greater amount of food consumed by the groups that underwent the obesity procedure compared with the reference groups [$F(1,6) = 75.80$, $P < 0.001$, $\eta^2 = 93\%$ of variance explained]. The marginally significant interaction between group and time was due primarily to the increased food consumption of the group that underwent the obesity procedure during the second block compared with the later blocks in the experiments.

There were no significant group or time effects (all F's < 1.00) or an interaction between group and time for fluid consumption (Fig. 3). Whereas the individually standardized measures for daytime activity decreased over time [$F(5,25) = 4.81$, $P < 0.01$, $\eta^2 = 49\%$], nocturnal activity did not [$F(5,25) < 1.00$]. There were neither statistically significant group differences nor interactions between time and group.

Covariance for food consumption per block with body weight or body weight with food consumption (two different sets of residuals) did not reveal any recondite main effects or interactions. In fact, the statistically significant differences between groups for body weight or food consumption were no longer significant statistically [$F(1,6) = 0.41, 0.78$, $P > 0.05$] if the shared variance with the other variable was first removed before the two-way analysis of variance was performed.

3.2. Individual analyses

The partial correlations between the proportions of weight gain (body weight subtracted from previous body weight and divided by the latter) per measurement and food and water consumption for that interval for each rat after the shared variance with nocturnal and daytime activity had been removed are listed in Table 1. All of the rats that underwent the obesity procedure displayed statistically significant positive correlations between the successive increments of body weight gain and food consumption during the previous (3–4 days) interval. Only one rat from the reference group showed this correlation.

Factor loadings for the five measures of change in body weight from the previous measurement (3–4 days), and water consump-

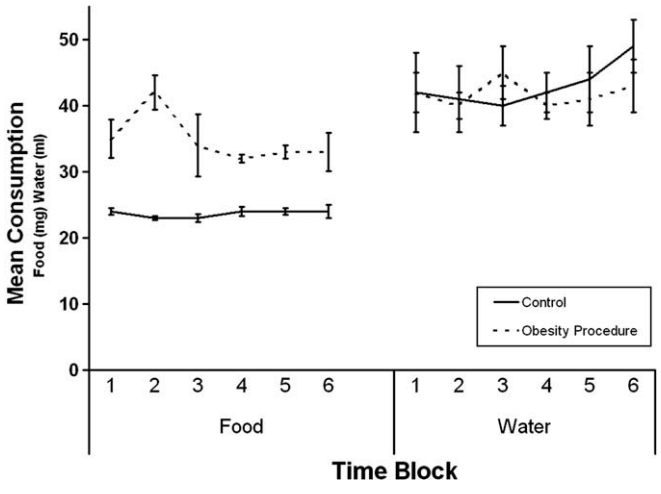

Fig. 2. Mean daily food consumption (in g) and water consumption (in cc) for groups that underwent the obesity procedure or that served as controls over the six blocks (18 weeks). Vertical bars are SEM.

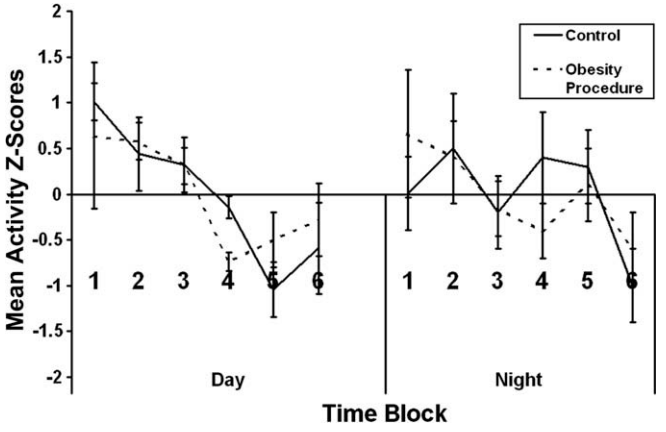

Fig. 3. Mean within-subject standardized (z) scores for daytime or nighttime activity for groups that underwent the obesity procedure and for the control group over the six blocks of time (18 weeks). Vertical bars are SEM.

Table 1
Partial correlations between relative increases in body weight compared to the previous measure and food and water consumption after removal of shared variance with night-time and day-time activity for each rat. Li/Pi/Ace (seized + acepromazine) refer to rats that received the obesity procedure; C = controls and Li/Pi/Ket (seized + ketamine) refers to rats that were seized but given ketamine.

Rat	Food	Water
Li/Pi/Ace 1	0.30[a]	0.52[b]
Li/Pi/Ace 2	0.30[a]	0.15
Li/Pi/Ace 3	0.46[b]	0.28
Li/Pi/Ace 4	0.40[b]	0.18
Li/Pi/Ket 1	0.03	0.34[a]
Li/Pi/Ket 2	0.54[b]	0.65[b]
Control 1	0.15	0.11
Control 2	0.02	−0.18

[a] $P < 0.05$.
[b] $P < 0.01$.

Table 2
Factor loading (correlation correlations) scores on the only two emerging factors (F1, F2) after varimax rotation for each of the eight rats for the five major behaviors.

	Li/Pi/Ace 1		Li/Pi/Ace 2		Li/Pi/Ace 3		Li/Pi/Ace 4		Control 1		Control 2		Li/Pi/Ket 1		4 Li/Pi/Ket 2	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Weight change		0.80		0.55		0.84		0.71	0.69			0.73		0.83		0.97
Food		0.59		0.84		0.79		0.86		0.79		0.73	0.78		0.91	
Water		0.83		0.79	−0.56	0.55		0.90		0.91	−0.70		0.85		0.91	
Daytime activity	0.94		0.93		0.84		0.91		0.88		0.85		0.62			0.81
Nighttime activity	0.94		0.93		0.85		0.93		0.92		0.99			−0.69		−0.79

tion, food consumption, daytime activity, and nighttime activity for the eight rats are listed in Table 2. For the rats that underwent the obesity procedure, change in weight loaded on the same factor that was associated with increased food and water consumption; activity loaded on a different factor. For the reference group, body weight was loaded on the same factor involved with activity for three of the four rats. Interestingly, body weight increases for both postseizure and ketamine-treated rats was associated with less nocturnal activity.

4. Discussion

It is well known that significant weight gain occurs during the month following either lesions [12] or electrical stimulation (kindling) [13] within the amygdaloid region in female rats. In fact, King et al. [12] concluded that obesity-inducing amygdaloid lesions involved the same subdivisions of the posterodorsal amygdala that mediate reproductive behavior. The seizure-induced damage from lithium/pilocarpine injections also damages this region [14], in addition to many other limbic and thalamic structures, particularly the nucleus reuniens, which has been shown to attenuate winter metabolic adaptations by causing an increase in body weight [15]. Lesions in tract systems that mediate information between the medial amygdala and ventromedial hypothalamus, such as the dorsal component of the stria terminalis [14], also facilitated increases in weight gain that were evident within about 2 weeks.

Our experiments [4] have shown that a single injection of lithium and pilocarpine, sufficient to induce limbic seizures, results in slow but continuous gains in body weight for months in only female rats if this procedure is instituted after infancy and before puberty and the rats are treated with acepromazine. Unlike many of the traditional means of inducing obesity [6,14,15] that result in conspicuous weight gains within a few days of the lesioning or stimulation, rats receiving the obesity procedure exhibit an insidious continuous weight gain for the rest of their lives.

What was not clear, until this study was conducted, were the factors contributing to this obesity. The results of the present study indicated that the difference in weight gain between the two groups is not related to diminished nocturnal or diurnal activity over about a 4-month period because these values did not differ. This appears to contradict the increased nocturnal activity compared with baseline for male rats following induction of the same types of seizures [7]. This may reflect a true gender difference or a transient phenomenon because these rats were observed during the 40 days after seizure induction, whereas in the present study, behavioral monitoring began more than 60 days after seizure induction (obesity procedure).

To accommodate the potential differences in cage equipment that measured the activity, within-subject *z* scores were calculated. There were no significantly differential slopes over time (as would be reflected in an interaction) for the two groups. Similarly, the rats that underwent the obesity procedure did not differ in their daily fluid consumption compared with controls.

The absence of concomitant increases in drinking for the rats that underwent the obesity procedure may not appear to be consistent with the frequent behavioral observation of postprandial fluid consumption. However, our data were based on averages over large (3-week) temporal blocks rather than individual eating episodes. Consequently, if concurrent increases in water consumption occurred during the eating but showed compensatory decreases afterward, these transients would not have been observed.

The normal water consumption by the rats that underwent the obesity treatment might be related to inflated estimates of food consumption because of nonconsumptive gnawing. However, this explanation would not accommodate the clear increases in weight gain. In addition, there was no evidence during the frequent and very careful cleaning of the cages to recover the food fragments of any qualitative differences in the manner by which the rats consumed food.

The rats that underwent the obesity procedure were heavier than the control rats by the time they were recruited for the study (body weights between 45 and 50 days of age did not differ between groups). The effects of the procedure accommodated more than 75% of the variance in body weight for the two groups. The interaction between time and group reflected the escalating increase in weight gain that was even greater than the normal weight gain over time for the controls. The group that underwent the obesity procedure also consumed more food. In fact, more than 90% of the variance in the food weight was due to group differences.

Symmetrical covariance by which the residuals for body weight and food or food and body weight were analyzed separately eliminated the significant difference between groups for these two measures. When one effect is removed and the other is maintained during symmetrical analyses of covariance or partial correlation, a causal direction is implied. When both effects are removed following symmetrical covariance, the most parsimonious explanation would be the involvement of a third factor with which both measures are strongly correlated.

One candidate would be microstructural alterations within the ventromedial hypothalamus of the rats that underwent the obesity procedure. Classic studies [16] have shown that damage in this region encourages extreme weight gain by persistent nibbling, shifts in the metabolic utilization of fats, and reduced locomotor activity. We cannot exclude the possibility that structures such as the core region of the nucleus accumbens [17], which is not damaged conspicuously in lithium/pilocarpine-induced seizures, are disinhibited. The present study suggests that the slow weight gains over their rats' lifetimes are related to this third factor and not to the fact that the rats were less active or drank less water.

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References

[1] Shimizu H, Mori M. The brain–adipose axis: a review of involvement of molecules. Nutr Neurosci 2005;8:7–20.
[2] Marquez AV, Farias ST, Apperson M, et al. Psychogenic nonepileptic seizures are associated with an increased risk of obesity. Epilepsy Behav 2004;5:88–93.

[3] Iriarte J, Parra J, Urrestarazu E, Kuyk J. Controversies in the diagnosis and management of psychogenic seizures. Epilepsy Behav 2003;4:354–9.
[4] St. Pierre LS, Persinger MA. Extreme obesity in one year old female rats in which seizures were induced before puberty by lithium/pilocarpine followed by a single injection of acepromazine. Epilepsy Behav 2005;7:411–8.
[5] Persinger MA, Bureau YRJ, Kostakos M, Peredery O, Falter H. Behaviors of rats with insidious multifocal brain damage induced by seizures following single peripheral injections of lithium and pilocarpine. Physiol Behav 1993;53:849–66.
[6] Loscher W, Brandt C, Ulrich E. Excessive weight gain in rats over extended kindling of the basolateral amygdala. Neuropharmacol Neurotoxicol 2003;14:1829–32.
[7] Persinger MA, Koren SA. Persistent elevation of nocturnal activity in rodents following apparent recovery from lithium/pilocarpine-induced limbic seizures. Percept Mot Skills 1998;86:1243–8.
[8] Persinger MA, O'Connor RP, Bureau YRJ, Parker GH, Peredery O, Zegil M. Synergistic induction of severe hypothermia (poikilothermia) by limbic seizures, acepromazine and physical restraint: role of noradrenergic alpha-1 receptors. Pharmacol Biochem Behav 2001;70:341–52.
[9] Ye SM, Edwards SJ, Rose RW, Steen JT, Clark MG, Colquhoun EQ. Alpha-adrenergic stimulation of thermogenesis in a rat kangaroo (Marsupialia, *Bettongia gaimardi*). Am J Physiol 1996;271:R586–92.
[10] Racine R. Modification of seizure activity by electrical stimulation: II. Motor seizures. Electroencephalogr Clin Neurophysiol 1972;32:281–94.
[11] Santi SA, Cook LL, Persinger MA, O'Connor RP. Normal spatial memory following postseizure treatment with ketamine: selective damage attenuates memory deficits in brain-damaged rodents. Int J Neurosci 2001;107:63–75.
[12] King BM, Cook JT, Rossiter KN, Rollins BL. Obesity-induced amygdala lesions: examination of anterograde degeneration and retrograde transport. Am J Physiol Regul Integr Comp Physiol 2003;284:R965–82.
[13] Bhatt R, Bhatt S, Rameswhar P, Siegel A. Amygdaloid kindled seizures induce weight gain that reflects left hemisphere dominance. Physiol Behav 2004;82:581–7.
[14] Purvis CC, Duncan MJ. Discrete thalamic lesions attenuate winter adaptations and increased body weight. Am J Physiol 1997;273:R226–35.
[15] Rollins BL, Stines SG, King BM. Role of the stria terminalis in food intake and body weight in rats. Physiol Behav 2006;89:139–45.
[16] Challet E, Maho YL, Malan A. Locomotor activity and utilization of energy reserves during fasting after ventromedial hypothalamic lesions. Physiol Behav 1995;58:257–64.
[17] Maldonado-Irizarry CS, Kelley AE. Excitotoxic lesions of the core and shell subregions of the nucleus accumbens differentially disrupt body weight regulation and motor activity in rat. Brain Res Bull 1995;6:551–9.